6/3/16

Friday, June 3, 2016
1:24 PM

Expression data - MG1655
Genomic data - MG1655
Strains in house - cloning strains (Top 10, DH5 alpha)

Cloning strains (i.e. DH5 alpha) - have knocked out restriction enzymes that won't digest your plasmid

Expression strains (i.e. BL21)

Wild type strains

1. Build
    a. When cloning, the transformation and ligation process doesn't result in your tube only having your desired plasmid (i.e. fragments)
    b. 1/100 efficiency
2. Cloning strain
3. Purify plasmid
4. 100% efficiency

Plasmid Purification
1. Centrifuge cells (form pellet at bottom) and remove supernatant
2. Lyse/extract
    a. 250 microliters of Buffer 1 (resuspension) - pipette into plastic 1.5 mL tube
    b. 250 microliters of Buffer 2 (lyses) - invert
    c. 250 microliters of Buffer 3 (neutralizes Buffer 2) - invert
    ○ DNA is going to be in the liquid, and the cloudy portions are going to be comprised of extraneous protein and whatnot
3. Spin/purify (10 min)
    a. Load supernatant (contains DNA) onto purification columns (silica) to which DNA binds
    b. Bind DNA
    c. Buffer A (inactivates enzymes to prevent DNA digestion - not necessary in cloning strains because they don't have those enzymes)
    d. Wash buffer
        - DNA is water soluble
    e. Elute w/ water

6/6/16

Monday, June 6, 2016
1:09 PM

BioBricks are identifiable by their specific BioBrick prefix and suffix sites that are restriction sites. This lends exellent versatility to those working with BioBricks because it allows them to cut BioBricks at specific locations and recombine them at ease.

We're going to be working with engineered E. coli - they have minimal genomes.

Chromosomal DNA and plasmid DNA have different markers (i.e. epigenetic methylation markers in chromosomal DNA) that allow them to be separately isolated.

Sanger Sequencing Primers

1. Primer length should be in the range of 18 to 22 bases
2. The primer should have a GC content of 50-55%
3. The 3' end of the primer should have a GC-lock
4. The melting temperature of any good primer should be in the range of 50-55 degrees Celsius
5. The primer should not include poly base regions (two in a row is fine, three is a bit risky, four is a no-go)
6. Four or more bases that compliment either direction of the primer should be avoided (the primer could anneal to either end of the DNA fragment)

Today!

- Pour more plates
- Make LB
- Order supplies
- Grow up more strain E. coli
- Autoclave glass beads
- Wash dishes, autoclave

6/7/16

Tuesday, June 7, 2016
1:17 PM

To do:

- The DNA coding for our intended protein (GFP - green fluorescent protein) does not have a promoter (DNA1)
- We have a promoter on a different piece of DNA surrounded by restriction sites (DNA2)
- Using EcoR1 and Xba1, we can cut DNA1 to open up the DNA strand right before the DNA corresponding to the protein
- Using phosphatases, we can remove the phosphates on the DNA bases at the sticky ends of where DNA1 has been cut. Since ligation requires the energy from the phosphate groups on DNA bases, this prevents DNA1 from reannealing
- Using EcoR1 and Spe1, we can cut DNA2 to remove the fragment containing the promoter
- Ligation will ideally have the excised promoter fragment ligate to the open DNA1 strand, though that is not the only fragment combination formed
- Heating up the fragments now at 80 C for 20 minutes denatures the restriction enzymes and stops the

- Using gel electrophoresis, we can isolate which DNA fragments are of the appropriate length, purify the gel (heat it up, melting the agarose, and isolate the DNA), and have our fragment!

Gel Purification Protocol

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6/15/16

Wednesday, June 15, 2016
12:17 PM

In our plasmid, the GFP DNA sequence comes right after the promoter. As the GFP molecules fluoresce, one can gauge how active the promoter is. However, GFP molecules have a tag at the end that causes them to degrade rapidly, which is necessary in order to provide a real-time representation of promoter activity (old molecules don't stick around, so only real-time protein production can fluoresce).

Golden Gate Assembly

- Digest
- PCR desired parts
- Leaves no scar!

6/20/16

Monday, June 20, 2016
2:28 PM

Today, we ran a gel electrophoresis experiment with the intent of moving on to gel extraction afterwards. The protocol for gel extraction entails running restriction enzyme digested plasmid DNA on an agarose gel with a voltage gradient. As DNA is negatively charged, DNA fragments will migrate towards the positive end of the gel and will separate by length & compaction because smaller and more tightly coiled DNA fragments can more easily traverse the agarose. Knowing what the approximate length of the desired digested DNA should be, one can gauge the different bands that result (visible under the UV light of the gel imager) and determine which band contains the appropriate DNA fragment.

The ensuing step requires cutting out the desired DNA from the gel so that the DNA can be isolated. DNA loss in this process is inevitable but the idea is to preserve as much of it as possible. Thus, it is ideal that the band be compact to minimize DNA loss and gel needing to dissolved during extraction. Unfortunately, the gel we ran today (1%, 100 V, 25 min) produced a DNA streak for the 14A and 14C samples. Having encountered this problem, we consulted one of the grad students in the Zhao lab group and he walked us through the gel cutting process. He also advised to try running a 2% at 120V for 20 minutes to create a more clearly defined and compact band.

6/21/16

Wednesday, June 22, 2016
12:01 PM

Using the recommended gel protocols proposed yesterday (2% gel at 120V for 20 minutes), we decided to run the 14A, 14C, and 1P DNA samples again. Despite our high hopes that the gel would produce tightly condensed, distinct bands, the same issue we had noticed with the previous gels persisted as the second bands for all three samples were diffuse streaks. We decided to proceed anyhow and used the gel cutting procedure shown to us yesterday to extract the appropriate DNA bands.

For 14A and 14C, we cut out the diffuse second bands because they correlated to shortest piece of digested DNA, which is exactly what we needed. For 1P, however, we cut out the first (well-defined) band because super-coiling of the plasmid actually allows it to be more compact than our desired fragment and move further along the gel.

We then made our way through the gel extraction protocol for 1P to gain some degree of familiarity with it. The protocol entails dissolving the gel surrounding the desired DNA fragments and, accordingly, the greater the gel volume the greater the volume of buffer needed to dissolve it all. For agarose gels 2% or lower, the protocol calls for 3 times the gel volume in buffer volume while gels greater than 2% require 6 times the gel volume in buffer volume. Given the diffuse DNA streaks for 14A and 14C, they had large gel volumes and would have required lots of buffer (which wouldn't be conducive to our limited gel extraction kit supplies). We called it a day after finishing the 1P gel extraction (took just under an hour) and stored the DNA in the –80 C with the 14A and 14C extracts.

6/22/16

Wednesday, June 22, 2016
8:30 PM

Today's work consisted almost exclusively of the weekly lab meeting, in which the following points were addressed:

1. Ordering primers - Caroline went through and double checked the primers that had already been uploaded to the drive and, after consulting Todd, also added a few bases to both forward and reverse primers to account for Golden Gate overhang.
2. Project ideas - different ideas for using our bacterial promoters were proposed, ranging from lycopene (determines the color of tomatoes) to insulin, culture smell, and extensions of the Oxford team's copper chelating agent bacteria.
3. I ran into Courtney in the morning and asked her about what was going on with the gels that we had been running and getting funky results on. Her recommendation was to use the agarose gels to visualize whether or not a band was present but then not bother with the process of gel extraction (lots of DNA loss). Instead, she recommended proceeding directly to PCR and amplifying the desired strand to such an extent that the concentrations of all the other DNA portions would be negligible.

6/27/16

Monday, June 27, 2016
3:13 PM

Today consisted of just the weekly student meeting.

Augustine started things off with an analysis of a synthetic biology article that can be found here: https://slack-files.com/files-pri-safe/T1GSTJPDY-F1L6PK0PM/acssynbio_2e5b00116.pdf?c=1467058203-91d83391647d1e3f13e01392d4f538782be2c7d1

We will be Skyping with the Oxford team to discuss our next steps later this week and will go through PCR tomorrow. Project ideas will also be culminating this week in a decision Wednesday/Thursday over which direction our project should go.

6/28/16

Wednesday, June 29, 2016
11:36 AM

Today we ran a PCR of the DNA we had for promoter 4202 (came from IDT as a pellet). After completing the procedure, we quickly ran a gel against a DNA ladder to gauge how long the amplified DNA was (thus also being a test of our forward and reverse primers). We found a prominent PCR DNA band at about the 400 base pair mark and since our strand was 352 base pairs in length + 10-15 base pairs on either side, we knew that the PCR had run successfully.

6/29/16

Wednesday, June 29, 2016
11:36 AM

Adviser meeting day! Huzzah!

Today everybody created and presented a slide on a possible application focus for our bacterial promoters and presented it to the advisers. We got some excellent feedback and are looking to move into the final stages of project selection.

7/1/16

Thursday, July 7, 2016
6:27 PM

Meeting with the Oxford Team!

1. They have a team of 11 people (almost twice ours)
2. They're keen on working with us and are looking at about the same timeline as we are as far as project completion goes
3. Lot of room for collaboration here

7/11/16

Wednesday, July 13, 2016
1:06 PM

7/12/16

Wednesday, July 13, 2016

1:06 PM

7/13/16

Wednesday, July 13, 2016
1:07 PM

Mariam and I met up in the morning to discuss ligating and cloning the gBlock construct

Adviser Meeting!

- Outreach Event - Sweetcorn Festival
  - Second weekend of first semester
  - Early Bird Registration for a nonprofit - $150 due by July 15th w/ application
    - Contact Katie for images, logos, etc.
    - Send email to coordinator asking about student RSO discounts
  - Corn DNA extraction?
  - Avoid GMO language
- Start fleshing out website and software ideas by next week
- Plate reader training
  - We should all get trained soon
  - Interlab kit should be arriving soon
- Cloning today/tomorrow, transformation tomorrow, finish up Friday

7/14/16

Sunday, July 17, 2016
5:17 AM

7/17/16

Sunday, July 17, 2016
5:17 AM

7/18/16

Monday, July 18, 2016
4:15 PM

Student Meeting

- We got accepted to the Sweetcorn Festival!
  - Use phrase "synthetic biology" rather than "genetic engineering"
  - Activities
    - Extract DNA from corn?
    - Extract DNA from human?
    - Genetic history of corn - how climate, movement across the world, utility (i.e. feed corn vs. sweetcorn), pesticides, and fertilizers have affected it
    - Some form of interactive game

        - Explaining promoters and efficiency - may be difficult due to the audience's lack of background
    - Photo Consent form? We'll need evidence to show iGEM
    - We'll be there both days of the festival and will likely only have a table to work with
    - Mariam says she remembers Courtney and Caroline saying that Outreach events don't have to directly relate to our project - can be generically tied to STEM
        - On the contrary, Augustine's look through the judging handbook indicates a synthetic bio and iGEM focus is wise
            - *Education & Public Engagement allows judges to reward exceptional outreach and education work.*
            - *It is not for "proselytizing" iGEM and synbio by telling the community that iGEM is great and will "save the world".*
            - *Great E&PE projects will focus on establishing a dialogue or sparking new scientific interest in the community relating to synthetic biology in general (vs. relating to a team's specific project).*
    - The College of William and Mary conducted a survey at their local Farmer's Market to gauge knowledge and concerns about synthetic biology
        - A survey sounds like an excellent idea - will mention at Wednesday's adviser meeting
- Quad Day
    - We're off the waitlist and will be there as an RSO!
    - A month and 3 days from today - August 21st
        - A week or two before the Sweetcorn Festival
        - Pamphlets we make for Quad Day can be reused at the Sweetcorn Festival

7/19/16

Wednesday, July 20, 2016
1:16 PM

7/20/16

Wednesday, July 20, 2016
1:16 PM

Weekly Adviser Meeting

- Sweetcorn Festival
    - Have an activity to engage kiddos while surveying parents
    - DNA extraction is a good idea for an activity, we should look online for more ideas
- Quad Day
    - Purpose is recruitment
    - Can talk about what we did to garner interest
- Interlab Study
    - Supplies should be coming in soon
    - Plate reader training w/ training
        - Grow cells for 24 hours and use plate reader to measure
            - OD (optical density - cellular mass, how much the cells have grown) - use clear plates so that light can pass through (the less the light that makes it through, the higher the OD)
            - Fluorescence - use opaque, black plates
            - Ratio of Fluorescence to OD

- If you're culturing in the wells in the plate reader, you can only have about 120-200 µL in each well and evaporation can happen over time, cells can conglomerate on the edges of the well while the plate is read in the middle, or the bottom of the well can be rounded or pointed (distorting fluorescence signal)
- Dilute yer sample to make sure that the plate reader can read the fluorescence value
  - Dilute cells in a buffer
- Washing your cells (twice!) helps to correct for autofluorescence
- Gather data in a tube (50 mL) or flask (25 mL) at 0, 2, 8, and 24 hour marks
- Look for opaque plates with clear bottoms? Otherwise (more likely), use two plates

- GFP/RFP
  - Use RFP in experiments as well - mCherry works well for E. coli

7/26/16

Tuesday, July 26, 2016
1:29 PM

We got the lycopene genes!

- They come already transformed in plasmids in E. coli that have been put into a tube with agar
- The agar is pierced with an agar stab in which there are cells
- We need to stick a pipette into the cell covered divot left by the stab, streak some LB plates, and let them grow overnight

7/28/16

Friday, July 29, 2016
3:21 PM

A lot happened today!

1.

7/29/16

Friday, July 29, 2016
3:17 PM

Augustine and I ran a Miniprep to purify the lycopene plasmid parts following their transformation yesterday.

- They are in two tubes labelled BBa_118013 and BBa_118004
- There is roughly 50 µL of eluted plasmid in each 1.5 mL centrifuge tube
- Labelled "Post-Miniprep" and with the date (7/29/16)
- Stored in the iGEM 2016 Miscellaneous DNA box

8/23/16

Wednesday, August 24, 2016
5:16 PM

9/28/16

Wednesday, September 28, 2016
10:24 AM

Meeting with Advisors

- Digestion yields have been very low
  - Starting with 200 ng, yielding 40 ng
  - It is ideal to start the ligation reaction (next step) with 100 ng
- Perhaps we are losing a lot in elution
  - Using water to elute can be tricky because it needs to be nuclease free and may not be at the appropriate pH
  - We should use the provided elution buffer
- Outreach
  - Low turnout at two weekends at Orpheum
  - Going to add another outreach event in the coming weeks